THE FRUIT SPOT OF APPLES

IMPERIAL BUREAU OF MYCOLOGY

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[Reprinted, without change of paging, from the BULLETIN OF THE TORREY BOTANICAL CLUB 35: 423-456, *pl* 29-35. 29 S 1908]

[From the Bulletin of the Torrey Botanical Club, 35: 423–456, *pl.* 29–35. 1908.]

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Charles Brooks

(with plates 29–35)

A considerable percentage of the Baldwin apples of New England are marred by the presence of certain fruit spots, described in the bulletins of the New Hampshire Experiment Station as the Brown Spot of Baldwins. The following paper is a report of the results obtained in an effort to determine the cause of this spot, its morphological and physiological characteristics, and means of prevention. A brief review of earlier work on this and closely related effects will be of value in the later presentation of the subject matter.

Review of literature

In 1879 Sorauer (1) described a disease which he called the "Stippichwerden der Aepfel." According to his description, brown or blackish brown spots developed on the surface of the fruit, extending into the flesh only .5 to 1.5 mm. These spots remained isolated and never involved the whole fruit. He found that they might remain without development for a long time and later develop rapidly in storage. He considered the spots to be due to a decomposition produced by a particular fungus and gave *Spilocaea Pomi*, previously described by Fries (2), as the probable agency.

Reichelt (3) reported a similar spot on apples but found that it was caused by a fungus belonging to the genus *Synchytrium*.

Frank (4) thought that the *Spilocaea Pomi* of Fries was a

sterile form of *Fusicladium dendriticum*, thus making the "Stippen der Aepfel" identical with scab.

Wortmann (5) made extensive studies of the "Stippen der Aepfel." He described the disease as producing numerous brown spots on the surface of the fruit. At first these were but 1 to 5 mm. in diameter and entirely separate and distinct, but later they might become larger and more numerous, practically covering the entire surface of the apple. The spots usually developed after the fruit had been gathered and while it was passing through its final ripening processes, but with especially susceptible varieties they sometimes appeared while the apples were still on the tree. The tissue beneath these spots was browned, possessed an overabundance of starch and in late stages was bitter to the taste. He did not consider the disease to be of fungous origin, not only because he was unable to find any trace of mycelium in the affected tissue but also from the fact that the spots were often covered by a smooth and unbroken epidermis and might be found at a depth of one centimeter from the surface and entirely separated from all other affected tissue. He believed that the disease was a physiological one and that conditions of transpiration were largely responsible for its occurrence. He found that varieties of apples that were susceptible to spotting had more lenticels and a thinner-walled epidermis than those less affected by the disease. On the other hand, these same susceptible varieties gave off less water in transpiration than the more resistant ones. From these observations and from the previously known fact that spotting might be prevented by a rapid drying of the fruit, he drew the conclusion that the extent of the disease was not determined by the actual amount of transpiration but by the readiness with which water was conducted from deeply seated cells to replace that lost in transpiration. The varieties that had the slowest rate of conduction were the ones that were most seriously affected. The concentration of the cell sap in the exposed tissue was the real cause of the injury and the acids and acid salts were the active agents in killing the cells. As an explanation of the fact that apples do not become spotted when dried very rapidly, he suggested that under such conditions the acid did not have time to act. He believed that the nature of the substances in the cell

sap partially accounted for the differences in the susceptibility of different varieties.

Zschokke (6) presented a detailed report in regard to the structure of the epidermis in core-fruits with special reference to the part it plays in determining their keeping quality. His description of the "Stippen" and his conclusions in regard to it are almost identical with those of Wortmann.

In Australia a disease supposed to be identical with the "Stippen" is described by Cobb (7) as Brown or Bitter Pit.

Craig (8) described a similar disease occurring in Canada as the Dry Rot of the apple. He found that sixty different varieties of apples were susceptible, but that the Baldwins were affected most seriously.

In the United States the spotting of apples has been repeatedly described and variously named. Selby (9) reported a disease of Northern Spies and other varieties which produced small brown spots just beneath the skin of the apple and which usually did not extend to any great depth into the tissue. He found no fungus present and considered it a breaking down of cells brought about by seasonal conditions.

Jones (10) described the "Brown Spot of the Baldwin Apple" as producing brown sunken spots the size of a pea or larger on the surface of the apple. The flesh underneath these spots showed a brown discoloration for an eighth of an inch or more in depth. The discolored portion was quite bitter to the taste. Specimens of the diseased fruit placed in a moist chamber soon developed small grayish pustules at or near the center of the spot, the pustule being from a sixteenth to an eighth of an inch in diameter. Specimens of the fungus were sent to J. B. Ellis for identification who reported that it was probably *Dothidea pomigena* Schw. Later, Jones (11) stated that the fungus occurring in the spots was quite different from the above species. In most cases, especially in the autumn and early winter, no fungus was detected in the browned tissue. When it was present it was quite obscure. The fungus was not identified because of inability to secure satisfactory fruiting specimens. He considered the fungus a saprophyte and of minor importance so far as the disease was concerned. In the later publication the spotting was given the following description:

"The disease usually appears superficially on the fruit as small sunken brown spots scattered over the surface of the apple, but more abundantly near the eye or apical portion. These spots may appear before maturity, but usually are seen only after the apples have lain in storage for some time, and tend thereafter to increase in number and size. The spots usually vary in diameter from two to five millimeters. The superficial spots usually lie immediately underneath the epidermis, which in the earlier stage of their development is unbroken. Upon cutting into such a spot it is found to consist of rather dry, dead and browned tissue, extending into the flesh for a distance about equal to its diameter. Similar areas of dead and brown tissue may occur scattered at various depths in the flesh nearly to the core. Examination shows the spots to be associated in their distribution with the occurrence of the vascular bundles of the fruit. The browned tissue may have a slightly bitter flavor in the older spots, but this bitterness is not constant and in no case in our observation is it very decided."

He found that while the spotting was worse on Baldwins than on any other variety it was quite common on Northern Spies and occurred on Greenings.

Stewart's (12) description of the "Baldwin Spot" is quite similar to that given by Jones. He did not detect the bitter taste that Jones had found the browned tissue to have. The smallest spots might show no brown color at all but be indicated merely by a deeper red color of the skin if situated upon the colored part of the fruit, or by a green color if situated upon the lighter portion. At the time the fruit was gathered the spongy tissue was found only underneath the surface spots, but after it had lain some three weeks in the laboratory many brown spots were found distributed irregularly through the flesh of the calyx half of the fruit, but not in the stem half. Apples placed in moist chambers showed no development of any fungus and pieces of browned tissue transferred to various culture media gave no growth. No beneficial results from spraying had been observed. In a later bulletin (13), an orchard was reported in which the disease had been almost entirely prevented by spraying. The most susceptible varieties were Baldwin, Northern Spy, and Rhode Island Greening.

Clinton (14) described the "Baldwin Spot" as showing first

in the fall as small sunken rotten spots on the surface of the fruit and later as isolated brown spots within the flesh, the tissue in these often collapsing.

In a later report (15) he described another disease of the apple which he called the "Fruit Speck." This disease showed superficially as small spots or specks scattered over the skin. These areas of brownish dead tissue usually varied from the size of a pinhead to a quarter of an inch in diameter and extended but slightly into the flesh. He could frequently see small ruptures at the center of the specks. Diseased tissue when placed in sterile culture media developed a fungous growth. The fungus in the various cultures was apparently the same. Talman Sweets were most seriously affected, Northern Spies suffered less, while Baldwins were injured but little.

Longyear (11) reported a disease common on Baldwins and other varieties in Michigan which he called the "Fruit Spot" of apples. He described it as appearing in the form of small, circular, slightly sunken spots of a brown color. The brown discoloration usually extended but a little way into the flesh of the fruit and the affected part possessed a bitter taste. The spots gave rise to spore-producing pustules. *Phyllachora pomigena* (Schw.) was credited as the cause of the trouble. Spraying as for scab was found greatly to reduce the disease.

Lamson (17) has published a number of reports on the "Brown Spot of Baldwins" as it occurs in New Hampshire. He gave the following description of the disease: "It is characterized by the appearance on the surface of the apple of numerous small brown spots, varying in diameter from a sixteenth to an eighth of an inch. The spots are slightly sunken or depressed so that the surface has a pitted appearance. They suggest the beginning of rot but do not increase in size ordinarily, though occasionally they do. The disease appears late in the season. The chief injury is to the appearance of the fruit. This is often so much damaged that otherwise perfect apples become seconds." He found that fungicides practically controlled the disease.

For the past two years the writer has been making a study of the spotting of New Hampshire apples. The results obtained are given under the various headings that follow. A knowledge of the host is necessary to an understanding of a disease.

THE STRUCTURE AND DEVELOPMENT OF THE APPLE

Epidermal structure. — The epidermis of the apple consists of a single layer of cells, the outer walls of which are strongly thickened. These outer walls consist largely of cutin, which as the apple matures is impregnated and covered with a resinous and waxy substance. The apple is thus furnished with a covering that at most points is practically impervious to water and fungi. Many unicellular hairs are found on the surface of the young fruit. The somewhat conical bases of these are inserted between the other epidermal cells. These hairs disappear when the apple is a few weeks old, but before this time the cuticle of the apple has considerable thickness. Zschokke (6) found that in dropping from the apple the hairs might break even with the outer edge of the cuticle, leaving their conical bases behind, but that more frequently they loosened themselves from the surrounding epidermal cells, leaving a deep scar, which, in the later development of the fruit, might become a definite break in the epidermis. The writer's observations agree with those of Zschokke. As late as the first of August these hair pits were quite common in the epidermis.

In the young fruit the stomata furnish such openings in the epidermis as are necessary for the passage of gases and liquids. They are very numerous early in the year and while the majority of them disappear later they are still quite common on the mature fruit. From three to four weeks after the fall of the blossoms numerous cork-like flecks begin to appear on the apple surface. The majority of these are lenticels (PLATE 32, FIGURE 4). When the epidermis of the young apple is broken, either through a natural or foreign agency, the opening is rapidly covered with cork cells. According to Zschokke (6) the lenticels develop as a result of breaks in the epidermis caused by the rapid enlargement of the apple, the stomata being the points that yield most readily to the strain. In a typical lenticel the cells are arranged in definite layers. In the apple the structure of these corky specks varies all the way from that of a typical lenticel to a few thick-walled cork cells promiscuously arranged beneath a minute break in the cuticle. Their early structure leads the writer to the opinion that while the majority of them develop at stomata others probably originate from the pockets left by the falling hairs.

The lenticels are far more numerous on the blossom half than on the stem half of the apple. This statement is based on actual count as well as on general observations. A square centimeter was marked off on the stem half of an apple and another on the blossom half and the lenticels counted on these areas. By averaging the results secured from ten Baldwins the ratio of seven to four was obtained as that existing between the number of lenticels on the blossom and stem halves of the apple. On Northern Spies the ratio was approximately five to three.

Hypodermal parenchyma. — Immediately beneath the epidermis are layers of cells that are distinctly different from those more deeply situated. These cells are smaller and more compactly arranged. They are oblong in shape with their greatest diameter parallel to the epidermis. They are rich in chlorophyll and take an active part in the nutrition of the apple. They contain the red coloring-matter of the fruit, which, according to Pick (18), develops from tannin under the influence of sunlight. There is a gradual transition from these cells to the large isodiametric ones that make up the mass of the apple tissue.

Vascular system. — The close relation of the conducting system to spot diseases makes a study of the vascular bundles of interest in this connection. The general distribution of these could be studied best in frozen apples. With these the soft flesh could be nearly all removed by holding the half of an apple under the current from a laboratory faucet. The remaining portion of the apple could be very satisfactorily studied by floating it in water. If an apple is cut into halves perpendicular to the core, ten green spots may be seen arranged in the form of a circle about midway between the core and the epidermis (PLATE 31, FIGURE 1). These are the large vascular strands of the apple. Smaller branches are given off from either side of them. FIGURE 7, PLATE 35 is a sketch of one of these ten vascular strands with the branches that arise from one side of it, *i. e.*, it shows about one twentieth of the vascular system of an apple. The main branches give off comparatively few smaller ones before reaching the margin of the surface zone of cells previously described. Here they branch profusely and anastomose in a seemingly indiscriminate manner. The veinlets from one large vein unite with those from another so that

the whole surface system is closely interwoven and connected. In the small veinlets the vascular elements become fewer and fewer, finally giving place to long narrow cells that seem to be transitional between the vascular tissue and that of the apple pulp.

Chemical composition. — The chemical composition of the apple varies greatly with the time of the year.

Pfeiffer (19) reported that crude fiber, ash, protein, sugar, acid, water, pectin, and dextrin all increased in the apple during growth.

Bigelow, Gore, and Howard (20) found that the sugar content of winter apples increased from the time of the June drop till November 5, when the apples began to break down and become mealy. During this time the acid, as estimated on a total solids basis, was constantly decreasing. These changes in the sugar and acid content took place very rapidly in the latter half of June and early part of July. The starch content reached its maximum before the last of July and rapidly decreased after that time.

Morse (21) found that the most important change in the apple in the "after-ripening" process was the change of starch to sugar. Cold storage retarded this and other chemical changes but could not prevent them.

Otto (22) reported that when ripe apples were allowed to sweat in piles the starch was entirely converted into sugar in two or three weeks, the fruit thus becoming more valuable for cider-making.

Zschokke (6) reported that the tannin content decreased in the ripening process. He found that the tannin was located largely in the surface cells of the apple. He believed that apples owed their resistance to decay fungi much more to the chemical composition of the cell sap, especially to the tannic and malic acid content, than to any mechanical protection.

THE FRUIT PIT OF APPLES

The writer finds that there are two distinct fruit spots that occur on New Hampshire apples. Some stages of either of these might be included under any of the previously mentioned descriptions. In the following pages one of these will be called the Fruit Pit and the other the Fruit Spot of the apple.

Characteristics. — In early stages of the Fruit Pit one finds numerous sunken areas from two to six millimeters in diameter on the

surface of the apple. These depressions are somewhat hemispherical in shape and have the appearance of bruises. At this stage the spots are not brown and often show no difference in color from the surrounding surface of the apple. They may be a deeper red than the adjacent tissue when occurring on the colored portion of the apple and a darker green when on the lighter parts. Later they begin to take on a brown tint, but at first this seems to show through from rather deeply seated tissue and not to arise from any discoloration of the epidermal or immediately underlying cells. Sections of such spots show that this is the case, and that the browning and the shrinking of the cells occur in the pulp of the fruit and in the tissue that is transitional between it and the hypodermal parenchyma. Later the surface cells also become dark brown. The epidermis may be smooth and apparently unbroken in both early and late stages. As the disease advances spots situated near each other often become confluent, developing into one large spot. In all such cases examined it was found that the original spots were closely connected with one vascular branch. The writer has been unable to detect a bitter taste in the browned tissue of the fruit pits.

Internal browning of tissue. — The surface spotting is often accompanied by browning of the tissue immediately surrounding the vascular bundles. Upon cutting such an apple one sees numerous apparently isolated brown spots. Further study shows that these are not isolated but are in reality continuous strands of brown tissue surrounding the vascular bundles. The portion of the vascular system that is most commonly affected is that lying within fifteen millimeters of the surface of the apple (PLATE 31, FIGURE 3). The surface spots often occur without the internal browning and also the internal browning may occur unaccompanied by any evident surface derangement.

Cause and occurrence. — Microscopical examinations of fruit pits have given no indication of the presence of fungi or bacteria. Brown tissue from the surface pits and from the more deeply seated vascular regions has been transferred to various culture media but always without securing bacterial or fungous growth. Both the fruit pit and the internal browning are evidently abnormal physiological conditions. Their nature and location would indi-

cate that they might be the result of some abnormal loss of water from the apple tissue.

The writer's observations give him no reason to conclude that the Fruit Pit is of common occurrence in New England. Within the last three years he has seen it on but one lot of New Hampshire apples. In this case the disease developed in cellar storage on some very large Baldwins that had been picked early in the fall. Frequent visits to the Boston markets for a study of spotted apples convince him that it is of rare occurrence on the fruit shipped to that city. Within the past year he has also made an unsuccessful search for specimens of Fruit Pit in the markets of Buffalo, Chicago, Toronto, and Montreal. He has recently had the privilege of making a study of the disease on apples from Maine, Michigan, and New York, from Ottawa, Canada, and from Cape Town, Africa. The specimens from all these sources had the characteristics previously given.

THE FRUIT SPOT OF APPLES

Occurrence and morphology. — This disease is very common in New Hampshire and in the Boston markets one can often find barrels of apples shipped from various sections of New England in which fifty to ninety per cent. of the fruit is spotted. It occurs on almost every variety of apple but is worst on the Baldwins, and the following statements apply especially to the conditions as seen on that variety. The disease appears about the middle of August. At this time one may notice spots of a deeper red on the colored surface of the apple and of a darker green on the lighter portion. They are but slightly sunken if at all and there is no suggestion of a bruise (PLATE 29, FIGURE 1). They usually occur at a lenticel but are sometimes covered with a smooth and apparently unbroken epidermis. The number on the blossom half of the apple is usually from two to ten times as great as that on the stem half. A part of this contrast might be accounted for by the difference in the number of lenticels on the two halves of the apple (see page 429), but must be partly due to some other cause. As the season advances the spots become more prominent (PLATE 29, FIGURE 2). On the red fruit surfaces they become more sunken and their color gradually changes from red to brown or black. At this

time they bear a close resemblance to the earliest stages of Black Rot. Sections of the spots show that the hypodermal parenchyma is affected from the first. Only in late stages does the browning and shrinking extend to the large isodiametric cells of the apple tissue. On the green surfaces the spots may become sunken before harvest time, but the depressions are due to a lack of growth and not to any shrinking of the flesh. A minute black speck usually develops at the lenticel and smaller specks may often be seen at a radial distance of one to three millimeters from the first. A microscopic study of the underlying tissue shows that the cell walls of the hypodermal parenchyma and transitional tissue are abnormally thickened and that this thickening is especially prominent in certain groups of brown cells that underlie the surface specks (PLATE 30, FIGURES 1, 2). In the center of these brown cell-masses one often finds small pockets produced by the collapse of one or two cells. In cellar storage the red spots become badly browned and sunken. The green spots may take a similar course but in many cases there is no marked change in their surface appearance. Under such circumstances, however, one often finds that the disease is spreading deeper into the tissue and that a pocket is being developed as a result of the shrinking of the cells (PLATE 30, FIGURE 3).

The development of the spots depends greatly upon seasonal and storage conditions. When the weather is damp and foggy during the last weeks before harvesting, the spots on the red fruit surfaces develop rapidly and become black and sunken before the fruit is removed from the tree. After gathering, the spots develop most rapidly on apples placed in boxes and barrels in cellar storage. On apples placed immediately in cold storage the spots make but little or no development. When apples are stored in a warm, dry place and wither rapidly, brown spots are not developed. On the withered fruit the green spots often stand above the surrounding portions, forming smooth green elevations that are in marked contrast with the yellow withered skin of the apple. Like the Fruit Pit the Fruit Spot is often accompanied by a browning of the vascular tissue. In late stages of the Fruit Spot one sometimes finds minute elevations at the lenticels in the center of the brown sunken areas. These seem to furnish the only distinguishing characteristic between the Fruit Spot and Fruit Pit at this stage.

It would be difficult to decide from the earlier descriptions given in the bulletins of the New Hampshire station (17) whether the Fruit Spot or the Fruit Pit was under special observation. The descriptions are better if taken as applying to the two diseases than if considered as applying to either to the exclusion of the other. The spraying experiments (17) were undoubtedly made upon the Fruit Spot. So far as the writer has been able to learn, a distinction between these two diseases has never been made.

An associated fungus. — As a result of spraying experiments made in the summer of 1906 the writer obtained data that agreed with those of Lamson (17) as to the value of fungicides in preventing the spotting of apples. Such results could be explained only by assuming that the disease was of fungous origin or that Bordeaux had some remarkable and undescribed effect upon the skin of the apple. The former supposition seemed far the more probable. As an initial test of the hypothesis, blocks of browned tissue were removed from beneath the epidermis of the apple and placed in sterile culture media. Agar and gelatin cultures in which the nutrient substance was furnished by a decoction of beets, beans, beef, or apples were tried with little but negative results. The growths upon the different bouillons were too varied to give any indication of a common fungus. It was noticed, however, that after sections of spots had been left in water for a few days they were overgrown by a fungus and matted together. The fungus was always the same and always started from the center of the spots. As a result of these observations liquid media were given a more thorough trial. Browned tissue was transferred to sterile distilled water and in four or five days the blocks were fastened to the bottom of the test-tube or Petri dish. In fourteen days a fungous mass six to ten millimeters in diameter had developed. Similar results were obtained with various bouillons, and while the growth was quite unlike in the different media, transfers from one to another proved that the fungus was the same in all. It was also found that the fungus had not been obtained in the agar and gelatin cultures because their surfaces dried too quickly to give it time to develop. Placing the cultures in moist chambers remedied this trouble, but the development was slower than that in liquid cultures.

This constant occurrence of the same fungus in the diseased tissue suggested that it might be the cause of the trouble. In view of the fact that fungi had been reported as being present in late stages of Fruit Spot, but had not been found to be present in earlier stages and again that no fungus had been reported as a factor in producing the trouble, one did not seem justified in drawing any conclusions until further studies had been made. It was important to determine whether the fungus is present in the earliest stages of the spots and whether it can be made to produce similar effects by artificial inoculation. Also, it was of interest to know from what sources the fungus can be obtained.

SOURCES FROM WHICH THE FUNGUS HAS BEEN OBTAINED

For an investigation of this phase of the subject some fifty lots of apples were tested and as many as six hundred separate cultures made. More than ninety per cent. of the cultures made from the green spots and about seventy per cent. of those made from the red spots gave a pure growth of the same fungus. More than fifty per cent. of the contaminations obtained from the red spots were due to *Sphaeropsis malorum* Berk. It was found that the fungus could be isolated from spots covered with a smooth and glaucous epidermis as well as from those having a lenticel in the center. The compound microscope revealed the fact, however, that the spots that seemed to be covered by an unbroken epidermis in reality had a stoma at the center.

In the summer of 1907 spots were tested for the fungus from their earliest appearance. The same fungus was obtained from these earliest stages as was found in the later ones. Cultures of the fungus were obtained from Talman Sweets and Gravensteins on August 21, and from Baldwins on August 28. In these as in all other cases the tissue for inoculation was removed from beneath the epidermis by means of a sterilized knife. At this time the spots had little or no brown corky growth beneath them.

Within the past two years the fungus has been isolated from apples obtained from Delaware, New York, Michigan, Maine, and Massachusetts, from Toronto and Montreal in Canada, and from the following points in New Hampshire: Webster, Durham, Lee, Wilton, Madbury, Walpole, Packer's Falls, Barrington, Deerfield,

Dover, and Nashua. It has been obtained from the following varieties: Baldwin, Greening, Northern Spy, Talman Sweet, Red Canada, Canada Baldwin, Red Astrachan, Fall Pippin, Bellflower, Gravenstein, Grimes Golden, Russet, Porter, Snow, Wealthy, Ben Davis, and Mann, besides a half dozen different sorts of native fruit. The spots on most of the above varieties are similar to those already described. On the native varieties and on the Talman Sweets the green spots are more common and such brown spots as occur usually have a bright red margin. The appearance on the Talman Sweets is quite similar to that described by Clinton (15) under the name of Fruit Speck. On Snow apples the spots are a deep black and have very definite margins.

LABORATORY INOCULATIONS

During the winter and spring of 1906–07 numerous inoculation experiments were made. The apples used in these experiments were first washed either in alcohol or in a five per cent. solution of formalin. Spores from pure cultures of the fungus were introduced beneath the epidermis by means of a sterile platinum needle. From four to twelve inoculations and an equal number of check punctures were made on each apple. These inoculated apples were placed in moist chambers to await developments. More than a hundred apples have been treated in this manner. Most of these were Baldwins, but Yellow Transparents, Manns, Astrachans, Red Canadas, Greenings, and Porters were also used. Baldwins removed from the culture chambers after two weeks time usually showed little or no contrast between the inoculations and punctures. The same condition was sometimes found at the end of three weeks. After four weeks time there was always a marked contrast (PLATE 31, FIGURE 2). In the punctures the needle path looked practically as clear-cut and fresh as when first made. The inoculations showed on the surface of the apple as brown sunken spots. A study of the underlying tissue showed that the cells around the needle path had shrunken and collapsed making a much larger opening than the original one. The tissue was browned for a radial distance of one to three millimeters (PLATE 30, FIGURE 4). When vascular bundles were near the needle path the browning extended several millimeters farther along their course

than in other directions. Free-hand and microtome sections of the brown tissue of the inoculations showed that but one fungus was present. There was a good growth of this, and its characteristics and its relations to the host cells were the same as are later described for the fungus in the pockets of the host tissue (see page 450). Upon transfer to culture media, inoculation tissue gave pure cultures of the above-mentioned fungus.

The rate of development of the inoculation spots varied greatly with the apple. All of the spots on a particular apple might be as fully developed at the end of two weeks as those on another apple, inoculated on the same day, from the same test-tube and placed in the same moist chamber, were in four weeks. Notes were taken on the acidity, dryness, and texture of such apples, but no conclusion could be drawn as to the cause of the difference in susceptibility of the different apples of the same variety. The results upon other varieties were the same as on the Baldwin except that on the Greenings, Yellow Transparents, Astrachans, and Porters the browning of the spot developed more rapidly. This fact would suggest that a soft tissue and a readily available water-supply are favorable to the rapid development of the fungus.

Attempts were also made to inoculate ripe unspotted apples by spraying spores over their surface and by dropping them into solutions that were full of spores, also by placing small pieces of spore-bearing agar on the lenticels. The apples were placed in a moist chamber and left until decay began. Only negative results were obtained in such cases. The apples used were Porters and Baldwins. Unfortunately, the above test has never been made when the apples were passing through the "after-ripening" stage.

FIELD WORK

From the theoretical as well as the practical standpoint it was of interest to determine when and under what circumstances the fungus gained entrance to the apple and how this infection could be prevented. Spraying experiments made in the summer of 1906 furnished some suggestions in this matter.

Fungicides. — The Bordeaux mixture used in these experiments was made with five pounds each of lime and copper sulphate to fifty gallons of water. The "K. L. B. P." was similar Bordeaux

with kerosene-limoid, as recommended by the Delaware Agricultural Experiment Station (23), containing 15 per cent. of kerosene. The plots consisted of five trees each. The following data were obtained by actual count of the gathered fruit.

Fungicide	Time of spraying	Percentage of picked apples spotted
None		99.7
Bordeaux	May 30	83
"	May 30 and June 8	69
"	June 2 and June 8	72
"	June 2 and June 21	42
"	June 21	56
K. L. B. P.	May 30 and June 8	86
K. L. B. P.	May 30 and June 21	39

These results show that the application made on June 21 was more effective than any other and would suggest that infection usually takes place after that time.

In the summer of 1907 further tests as to the time of infection were made. The Bordeaux used was of the 3–3–50 formula. As the fungus had been found to make a poor growth in alkaline solutions it was thought advisable to try the effect of lime as a spray. Five gallons of lime were added to each fifty gallons of water for this mixture. A resin-lime-solution was also used. It was prepared by adding five pounds of lime and five pounds of resin fish-oil soap to fifty gallons of water. As it was not known that the fungus was the primary cause of the trouble, it seemed possible that some compound with little or no fungicidal value, but which would stick to the fruit as well as the Bordeaux, might have a hardening effect upon the epidermis and thus decrease the spotting. Accordingly calcium phosphate was formed as a precipitate by mixing dilute solutions of lime and sodium phosphate. Two and a half pounds of lime and five pounds of sodium phosphate were added to each fifty gallons of water. The plots consisted of five trees each. The following results were obtained by actual count of the spotted and unspotted apples on the trees at the time of gathering.

Mixture	Time of spraying	Percentage of picked apples spotted
None		33.8
Bordeaux	June 17, June 24, and July 9	1.1
"	June 25 and July 9	1.5
"	July 9 and July 27	1.7
"	July 27	20.4
Lime	June 25 and July 9	16.7
Resin lime	June 25 and July 9	12.9
Calcium phosphate	June 25 and July 9	23.4

The disease was not so serious as the preceding season, but the results were just as marked. While the lime was beneficial and the resin lime even more effective, it can be seen that both were far inferior to Bordeaux. The calcium phosphate was as evident on the foliage and fruit at the close of the season as the Bordeaux, but it seemed to have had but little effect upon the disease. If it had any effect upon the skin of the apple it could not be detected. A study of the results obtained from the use of Bordeaux shows that the application made on July 9 was more effective than any other. It also indicates that applications made as late as July 27 may materially reduce the disease. These facts will be of special interest when viewed in connection with the results obtained in orchard inoculation experiments.

Inoculations. — Throughout the summer a large stock of liquid flask-cultures was kept on hand. The spores from these were added to water or to a one per cent. sugar solution and applied to the trees by means of a bucket-pump spraying outfit. The cultures used were approximately of the same age and the number of them added to a given quantity of water was always the same. The sprayings were all made between four and six o'clock in the afternoon. At each time two trees were thoroughly treated with water containing spores and two others with the sugar solution. These trees were given a second spraying two or three days later. The next week other trees were treated in the same manner. All of the trees were Baldwins. It was not considered necessary to spray the check trees with sterile water as the applications made to the other trees did not amount to more than an ordinary dew, and dews were common during the time of making the experiments. The data secured showed no contrast between the trees sprayed with water and those treated with the sugar solution. The results

are given in the first of the tables below. The data were obtained by actual count of the apples at harvest time.

Only one bearing Baldwin tree was convenient to the laboratory. Inoculation experiments similar to those described above were made on the limbs of this tree. No sugar solution was used, however, and the spores were applied to the individual apple by means of an atomizer. Some half-dozen limbs bearing from six to twelve apples each were treated at a time. The limbs were selected from different portions of the tree so as to eliminate the factors of light and moisture as far as possible. Only one set of limbs was sprayed more than once and this was treated twice a week throughout the season. The sprayings were all made between four and six o'clock in the afternoon. The sprayed apples were not covered or protected in any way. Although the tree was large it is readily seen that plenty of opportunity must have been given for the spores to be carried by the wind from the inoculated apples to others near them. The results obtained from this experiment are given in the second table below.

I

Trees sprayed between	Percentage of picked apples spotted
July 5 and July 27	70.4
July 27 and August 10	43.5
August 10 and September 12	38.9
Checks	33.8

II

Limbs sprayed between	Average number of spots per apple
July 2 and July 15	15
July 15 and July 31	13
July 31 and August 15	11
July 2 and August 15	28
Checks	9

The results varied greatly with the individual limb and tree. It was intended to compare the data obtained with the weather records but it was found at the close of the season that these were in an unsatisfactory condition and could not be relied upon.

Inoculation experiments similar to the above were carried on in the summer of 1908 and results equally as striking were obtained. In spite of the very dry weather in July, inoculations made in that month were far more effective than those made in August.

Other inoculation experiments were made by tying sheets of rubber closely around Baldwin apples and filling the bags thus formed with water that contained an abundance of spores. Check apples were similarly treated with water without the addition of the spores. These sacks were left on the apples three days, and then removed late in the afternoon. The inoculations made in July increased the number of spots by more than a hundred per cent., and those made in August showed a very definite increase over the checks.

One would seem to be justified in concluding from the sets of data above that the possibility of infecting the apples with the fungus in question decreases from July 5 to the close of the season and that the month of July is the time when the majority of the infections naturally occur.

On August 30, September 7, and September 12, 1907, attempts were made to inoculate Baldwins and Northern Spies by introducing the spores under the skin of the apple by means of a sterile platinum needle. Six inoculations and six punctures were made on each apple. The apples were left on the tree till gathering time, October 10. A hard corky growth developed around the needle path in both punctures and inoculations. No difference could be seen in the two at the time of gathering and no change in either was apparent in storage. Tissue from the inoculations when transferred to culture media either gave no growth or a growth of bacteria. The fungus was evidently unable to develop in the column of cider that must have filled the needle path after inoculation. This fact is in agreement with data given later showing that the fungus did not develop on Baldwin cider made from apples gathered the last of August, even when this was diluted to one third its original strength.

CHARACTERISTICS OF THE FUNGUS

In order to learn as much as possible of the nature and identity of the fungus it has been grown upon a large number of culture media. Except where otherwise stated the decoctions used were prepared in the manner prescribed in bacteriological and pathological texts.

In all liquid cultures, except with a few very unfavorable

media, the fungus began its growth in the bottom and on the sides of the vessel. After four or five days the entire surface that was under water was thickly dotted with minute colonies. (This was not true of the sides of Erlenmeyer flasks nor of the upper wall of a slanting test-tube.) The number of colonies was dependent upon the amount of surface and the number of spores introduced rather than upon the quantity of solution. These colonies soon developed into hemispherical gelatinous masses that could not be crushed when placed on a slide under a cover-glass because of the readiness with which they slid from between the glass surfaces (PLATE 31, FIGURE 4). A microscopic study of the material showed that its gelatinous nature was due to the fact that the hyphae upon coming in contact with each other became fastened together. This attachment often extended to a breaking down of the walls between the hyphae and the merging of two cells into one. The result was a peculiarly anastomosed fungous mass. Spores were most abundant on the surface of these masses but were produced throughout the colony. They were hyaline, consisted of from one to five cells and were from 2 to 3 μ wide by 20 to 60 μ long. The hyphae were septate and about 3 μ in diameter. The spores were cut off from the tip of knob-like projections on the side of the hypha as described below for the agar cultures. A few days later these colonies would go to pieces and a growth would soon begin to form on the surface of the liquid. This growth was light-colored at first and of uniform texture throughout, but later had a very definite zonation both as to color and structure (PLATE 33, FIGURE 2). On the surface was a layer of hyaline conidiophores arising from a zone of vertical brown hyphae that formed the upper margin of a dense black stromatic layer. The hyphae of this stroma were thick-walled, abundantly septate, and from 3 to 6 μ in diameter. Beneath it was a less compact layer composed of a mixture of coarse and fine threads. In some cases there was a series of such zones as have just been described. This may have been due to the fact that the liquid had been left on the top of the stroma when examinations of the flask were made. In a five months old flask culture it was noticed that numerous, somewhat hemispherical elevations had developed on the surface of the stroma (PLATE 33, FIGURE 1).

An examination of cross sections of these showed that numerous U-shaped cavities had developed on their surfaces and that where these were present the layer of conidiophores was wanting. These cavities were bordered by rather dense layers of mycelium and contained parallel erect hyphae with thinner-walled and almost isodiametric cells that in some cases gave a suggestion of pre-sporogenous tissue.

In agar cultures the conidia were produced beneath the surface of the agar. One spore would be produced and pushed aside to give way to a second, and this followed by a third and so on indefinitely (PLATE 35, FIGURE 2). In young cultures nearly all of the mycelium was beneath the agar, later a mass of coarse aerial hyphae developed (PLATE 32, FIGURE 5). In such cases a black stromatic layer was formed just beneath the surface of the agar.

Chlamydospores were common in all old cultures.

Conidia germinated rapidly in hanging-drop cultures (PLATE 34, FIGURES 1, 2, 3). Under similar conditions chlamydospores germinated as shown in PLATE 35, FIGURE 4. Each cell of the stromatic mass in old cultures seemed to have the power to send out hyphae when transferred to a fresh medium (PLATE 35, FIGURES 5, 6).

The conidia from very old cultures did not germinate but the chlamydospores and thick-walled hyphae retained their vitality a long time. Germination was secured from chlamydospores in an agar culture that was twenty-six weeks old and in which the medium had been hard and dry for more than five months.

The fungus developed as well in cultures at a temperature of 15 degrees as at 20 degrees but made a poor growth at 30 degrees. It was killed by an exposure for five days to a temperature of 37 degrees. It was evidently not injured by prolonged exposure to low temperatures, as it was repeatedly isolated from apples which had been in cold storage for several months. It was also obtained in culture from an apple which had been exposed for eight days to a temperature varying from — 28 degrees to — 6 degrees C.

RELATION OF THE FUNGUS TO NUTRIENT MEDIA

The fungus grew best on acid media and was very sensitive to sugar in culture solutions. In most culture media that lacked

sugar the fungous growth was white or pink, but where sugar was present as the principal ingredient it was olive or black. The solution given below was found to give a very satisfactory growth of the fungus and was used for all stock cultures. For the sake of convenience it will be referred to hereafter as solution *A*.

Glucose or sucrose	10 grams
Apple bouillon	25 c.c.
Sodium chloride	1 gram
Liebig beef-extract	1 gram
Peptone	2 grams
Water	875 c.c.

The following notes give some of the characteristics of the fungus in various culture media.

In water. — Blocks of infected apple tissue two or three millimeters in diameter gave a good growth of the fungus when dropped into sterile tap or distilled water. In less than a week the fungus had fastened the blocks to the bottom of the test tube and later spores were produced in limited numbers. The hyphae were about 2 μ in diameter, hyaline, and sparingly septate. Spores germinated readily in distilled water, producing hyphae several hundred μ long in a few days.

In peptone beef bouillon. — In this medium the growth was a pinkish white, beginning as colonies on the walls of the flask and later forming a shiny pink layer on the surface of the liquid. There was a fair production of spores. The hyphae were coarser and shorter than those described above.

In glucose bouillon. — Olive colonies developed on the side of the test-tube, followed by a dark olive growth on the surface. Spores were produced in abundance and were always hyaline. The hyphae were thicker-walled and more abundantly septate than in other media.

In solution A (see above). — The growth was more rapid and the spores were produced in greater abundance than in glucose bouillon. The hyphae were hyaline except in the surface growth of very old cultures.

In milk. — The fungus developed only on the surface of the milk. After seven days a slimy pink band of fungous growth would be seen on the walls of the test-tube and a layer of brown

whey two to five millimeters deep would be found on the top of the milk. The remainder of the medium was unchanged and the whey was separated from the milk by means of a film-like layer of curd. At the end of fourteen days the whey had a depth of ten to fifteen millimeters and in three weeks had often nearly reached the bottom of the test-tube. The layer of curd always separated the milk from the whey and in old cultures formed a solid white mass several millimeters deep in the bottom of the tube. Cultures in fermentation-tubes showed that no gas was produced. The hyphae as developed in milk were short, thick, and much branched. Spores often produced other spores by a sort of budding process.

In peptone potato bouillon. — The fungus made a good growth. No browning of the solution was evident in nineteen days but at the end of two months it was browned to a depth of two centimeters and later was browned throughout.

In peptone corn-starch bouillon. — The results were exactly as obtained in peptone potato bouillon except that the browning developed more rapidly.

In potato-starch bouillon. — The fungus made a fair growth. The hyphae were hyaline. The solution was not browned.

In corn-starch bouillon. — The growth was like that in potato-starch bouillon except that in old cultures the hyphae became olive.

In apple bouillon. — Though the tubes were repeatedly inoculated, the fungus did not develop. The bouillon was made from Baldwins gathered the last of August.

On apple cylinders in water. — The fungus made a fair growth. The hyphae were coarse and of a dark olive color. But few spores were produced. The apple tissue became brown in old cultures, a thing which did not happen in the check-tubes.

On potato cylinders in water. — A black slime developed, upon the surface of which were tufts of hyaline hyphae. The spore production was much like that obtained in milk.

On beef bouillon gelatin. — The development was very slow when surface inoculations were made. Liquefaction was scarcely evident in seven days but developed more rapidly after that time. It was crateriform in character. From three to eight weeks were

required for complete liquefaction. In old cultures the liquid was slightly browned. In stab cultures the growth was arborescent [FIGURE 1 (*text*)].

On beet bouillon gelatin. — The hyphae were darker than those in the beef gelatin and the liquefied gelatin was brown from the first.

FIGURE 1. Stab culture of *Cylindrosporium Pomi* on beef bouillon gelatin.

FIGURE 2. Stab culture of *Cylindrosporium Pomi* on beet agar.

On apple bouillon gelatin. — The development was slower than in other gelatins. The fungous mass was olive-black. The gelatin was liquefied and browned.

In beef bouillon agar. — The surface growth was umbonate. It was pink in color and had the appearance of a bacterial culture. Later the growth sometimes became darker at the margins. The hyphae were abundantly septate and the cells often swollen to circular form and to several times their usual width. Typical spores were not produced.

On glycerin agar. — The appearance of the colonies was similar to those on beef agar but they soon turned to an olive color. The hyphae appeared normal and spores were produced in abundance. But few aerial hyphae developed.

On maltose agar. — The colonies were pink at first but later were covered with hyaline aerial hyphae. The development was slow and but few spores were produced.

On beet agar. — In stab cultures the growth was umbonate, and arborescent [FIGURE 2 (*text*)]. The hyphae were hyaline with the exception of an olive mass on the surface in the center of the colony. Spores were produced beneath the agar.

On solution A agar. — The growth was entirely beneath the agar at first. Later an olive stromatic mass developed at the surface and from this were produced numerous aerial hyphae. The spores were abundant and were produced beneath the surface (PLATE 32, FIGURE 5).

Miscellaneous media. — Besides the above media the fungus has been grown on various mixtures and under various conditions in an effort to produce other fruiting forms. Among the substances used were rice, ground whole wheat, hominy, peptone, corn stalks, and various phosphate and potash solutions. On rice and hominy the growth was white on the surface but olive beneath. On ground whole wheat it was black throughout and gradually turned the wheat to a deep brown. In all peptone mixtures the culture medium was browned, the greater concentrations being changed most in color. Sodium ammonium phosphate added to a sugar solution caused a slimy pink growth to develop instead of the usual coarse olive fungus. This was not true of potassium phosphate.

The above culture notes show that while the fungus is responsive to changes in food material it is able to make some sort of growth on almost any medium.

Acidity. Enzymes. — Since the acidity of the cell-contents of the apple had been considered an important factor in the production of the "Stippen," it was thought advisable to test whether the fungus in question increased or decreased the acidity of culture media. Thirty 100 c.c. flasks were thoroughly cleaned as for physiological culture work and 50 c.c. of solution A added to each. After sterilization twenty of these were inoculated with the fungus. All thirty of the flasks were kept under the same conditions for sixteen days. At this time the acidity of the inoculated flasks and those not inoculated was determined by titrations. The large amount of sugar present reduced the sensitiveness of the indicator to such an extent that no definite results were obtained. There had at least been no marked change in acidity.

Neutral litmus milk-tubes were inoculated and were watched for any indication of acidity. The lavender color disappeared only to give place to the brown of the whey.

Some culture material which had originally been solution A but which had had the fungus growing on it for more than five months, produced but an extremely scant growth after sterilization and reinoculation. Tests were made to determine whether the reduction in growth was due to the production of some harmful substance or to lack of food material. 25 c.c. of this used solution were placed in each of fourteen 100 c.c. flasks. To two of these was added as much of each of the original food materials as had been added to an equal quantity of water in the beginning. In another two the peptone content was increased as described above but the solution left otherwise unchanged. The other food constituents were added to other flasks in a similar manner, and two flasks were left unchanged as controls. The flasks were all inoculated with the usual fungus. The ones to which all the original constituents had been added gave a luxuriant growth of the fungus. The flasks to which sugar had been added came next in amount of development and those in which the apple content had been increased came third. The addition of peptone increased the growth but the flasks to which beef extract and sodium chloride had been added gave no better development of the fungus than was obtained in the controls. These results would suggest that the fungus failed to make the usual development in old culture media because of lack of food material rather than from any harmful compound produced, and also that the acidity of the solution must have been decreased by the growth of the fungus. Beef extract and sodium chloride were evidently not used by the fungus in the quantity in which they were added to solution A.

Efforts were made to determine whether the browning that accompanied the fungus in the apple tissue and also in various other culture media was due to an enzyme or other product of the fungus. Strips of sterile uncooked apple tissue were dropped into the old solution A previously described and also into a similar but fresh solution. After three weeks the tissue was unaffected in both of these. This old solution was also added to tubes of milk but no change in color was apparent. Some of the old culture solution

was passed through a Chamberland filter and its effects upon apple tissue tested, but with only negative results.

The toxic effect of malic acid and tannin on the fungus was tested in Van Tieghem cells. Germination was entirely inhibited by a .5 normal solution of malic and greatly retarded by .125 *n* solution. The fungus made a fair growth in the latter solution. With tannic acid, germination was inhibited by a .025 *n* solution and only an abnormal growth was made in .0125 *n* solution. The fungus gave a fair growth in .00625 *n* solution.

According to Alwood and Davidson (24), Baldwins have .039 gram of tannin and .68 gram of acid as malic to each 100 grams of juice from the ripe fruit, *i. e.*, the juice would be about .002 *n* solution of tannin and about a .1 *n* solution of malic acid. These data agree with the fact that the fungus makes a fair growth on ripe apples. They show that any large increase in the acidity of the apple would prevent the development of the fungus in a cider culture. The tannin in the surface zone of the apple is probably greater than the above data would indicate, as tests made by the writer as well as the work of Zschokke (6) show that the tannin content in the hypodermal parenchyma is much greater than in the more deeply seated tissue. It is interesting to note in connection with the extreme sensitiveness of the fruit spot fungus to tannin that Alwood and Davidson (24) found the Baldwin apple to be extremely low in tannin content.

THE RELATION OF THE FUNGUS TO THE HOST

In connection with the other work upon the disease a microscopic study was made of the fungus as found in the tissue of the spots. In the preparation of material several killing agents were used, among these were absolute alcohol, various strengths of chrome-acetic, weak Flemming, Carnoy's fluid, and picro-acetic. The last two were found to be far the most satisfactory. Their superiority lay in the fact that they did not cause the epidermal and closely related cells to become so hard and brittle as the others did. Various contrast stains were tried on the tissue but none found more satisfactory than Delafield's haematoxylin followed by erythrosin. Both the apple tissue and the older threads of the fungus held the haematoxylin

quite persistently. The best results were obtained by staining fifteen or twenty minutes in haematoxylin, washing in acid alcohol until the stain had almost disappeared, transferring to water and then to erythrosin and leaving the slide in the latter stain for several hours. Erythrosin gave fair results when used alone. Free-hand sections were found very serviceable but serial microtome sections were generally used. Various thicknesses of sections were tried. Thin ones were best for a study of the stomata and lenticels, but sections 40 to 60 μ thick were found more satisfactory in tracing the mycelium of the fungus. This is not surprising when we bear in mind the large size of the apple cells and extreme fineness of the mycelium.

Every form and stage of the disease was studied. More than a hundred spots were sectioned and the fungus was found in every spot. This was true of the spots located at stomata and covered by a smooth epidermis as well as of those situated at the lenticels. In the younger stages the fungus had made very scant growth. In several cases it was actually identified in but one or two places in the entire series of sections and these possibly a millimeter or more apart. The threads were hyaline, granular, and apparently non-septate. They were extremely fine, in some cases being less than 1 μ in diameter. They had exactly the same appearance in these young stages of spots as when grown in extremely dilute solutions.

A study of the later stages showed that the fungus had accompanied the browning of the tissue in its spread. In the lenticels of the red spots one sometimes found a band of thick-walled promiscuously arranged cells passing through the organized layers of the lenticel, thus connecting the browned tissue beneath with the break in the epidermis above (PLATE 32, FIGURE 1, and PLATE 34, FIGURE 4). In such cases the fungus was present in both the band of cells and the more deeply seated shrunken tissue. In the green spots the fungus was found both in the groups of brown cells beneath the lenticel and in those a short distance from it (page 433). In many cases it seemed to have remained encysted in the center of these groups of thick-walled cells (PLATE 30, FIGURES 1, 2). In others it had broken through them, spreading deeper into the tissue, browning and killing the cells along its

course (PLATE 30, FIGURE 3). In no case was the fungus definitely traced from one of these pockets to another. Careful search was made for hyphae that had penetrated the cell-walls but none were found. In some cases they seemed at first sight to be within the cells, but a closer observation showed that the cells had collapsed and that the hyphae were in the cavity they had left. The mycelium in the pockets of the host tissue was coarse, septate, thick-walled, and brown, such as was often obtained in the stromatic layers of cultures. From these coarse threads arose fine hyaline apparently non-septate ones which spread out into the apple tissue. Chlamydospores were common on the coarse hyphae but conidia were never found within the apple tissue.

As was previously mentioned, spots that had become much enlarged, sunken, and browned sometimes had a minute elevation in the center (page 433). Sections through these showed that the epidermis had been raised in this manner through the agency of a fungous mass beneath. A dense stroma from 60 to 100 μ in diameter and about 50 μ deep, occupied a pocket beneath the lenticel or stroma. Equally dense layers extended laterally from this between the host cells for a radial distance of 100 to 400 μ. The stromatic mass was hyaline or of a yellowish tint, and was composed of closely woven, septate, thick-walled hyphae that had a diameter of about 5 μ. In spots in which the fungus had not yet broken through the epidermis a somewhat conical fungous mass that seemed to be composed of fine granular hyaline threads arose from the upper surface of the stroma (PLATE 32, FIGURE 2). In older stages this had been forced through the epidermis as a layer of erect hyaline sporophores (PLATE 32, FIGURE 3). The sporophores were extremely thin-walled, usually septate, and either branched or unbranched. The conidia were produced in a manner similar to that already described for the fungus in cultures (PLATE 35, FIGURE 2). They were hyaline, from one- to five-celled, 2 to 2.5 μ in diameter, 15 to 70 μ long, often larger at the basal than at the free end, and were variously curved and contorted.

IDENTITY OF THE FUNGUS

The descriptions of the previous pages together with the various figures to which references have been given furnish

a fairly complete morphology of the fungus in question. In young stages of fruit spots and in all dilute solutions the hyphae are hyaline, granular, apparently non-septate, and are from 1 to 2 μ in diameter. In more concentrated solutions they are usually granular and hyaline, have a diameter of 2 to 3 μ, and are broken up into cells having a length of 30 to 100 μ. Stromatic hyphae and those in the pockets of the host tissue are various shades of yellow and brown in color and are composed of thick-walled, somewhat barrel-shaped cells about 5 μ in diameter and 6 to 20 μ in length. Chlamydospores are a common accompaniment of these coarse threads. They are brown, thick-walled and have a diameter of from 4 to 6 μ. In young liquid and agar cultures conidia are produced from knob-like projections on the side of long, branching, septate, apparently vegetative hyphae. Many conidia are produced from a single one of these projections. The conidia are hyaline, granular, one- to five-celled, from 2 to 2.5 μ in diameter and from 15 to 80 μ in length. In outline they are straight, curved, or sigmoid. They germinate rapidly, sending out one or more hyphae from each cell. In some media the germinating spores produce other spores directly without the development of a mycelium. In old cultures a stroma is produced from which arises a layer of hyaline conidiophores. They are from 20 to 60 μ long and differ from the vegetative hyphae but little save in a reduction in length and branching. The conidia and their manner of production are similar to that described above. Pustule-like cavities having no special peridium develop at stomata and lenticels beneath the epidermis. Later they rupture the epidermis, exposing a layer of hyaline, septate, sparingly branched conidiophores. The conidia are produced from knob-like projections on the conidiophore and have the characteristics given above for spores produced in culture media.

The structure of the pustules and of the spores places the fungus in the genus *Cylindrosporium*. Of the species in this genus enumerated in Saccardo, it bears closest resemblance to *Cylindrosporium Ranunculi* (Bon.) Sacc. This fungus was isolated from leaves of *Ranunculus acris* and *Ranunculus bulbosus* in Italy. So far as can be determined, it differs from the apple fungus in the manner of freeing its spores and probably also in the structure of

its sporophore. From these facts and from the unlikeness of the hosts the writer is not inclined to consider the two fungi identical. For the Fruit Spot fungus he suggests the name *Cylindrosporium Pomi.*

Cylindrosporium Pomi Brooks, sp. nov. — Acervulis pallidis, subeffusis, primo subepidermicis, dein erumpenti-liberis. Conidiis hyalinis, granulosis, filiformibus, rectis vel flexuosis, 15–80 $\mu \times$ 2–2.5 μ. In fructu *Mali Mali* (L.) Britton, in America Boreali.

SUMMARY AND CONCLUSIONS

1. The writer considers that the facts and indications given justify the conclusion that the Fruit Spot of New Hampshire apples is due to a parasitic fungus, *Cylindrosporium Pomi* Brooks.

2. This fungus gains entrance to the apple in July or early August, a time when the stomata are being torn open and the protecting layers of the lenticels are not yet formed, a season when the metabolism of the apple is extremely great and the transpiration stream necessarily large.

3. The fungus makes its way into the intercellular spaces beneath the stomata and between the cells of the surface zone, obtaining the substances necessary for its existence from the transpiration stream and from the rapidly maturing host cells.

4. If the fruit is attacked before the cells have lost their power to respond to external stimuli the fungus is soon partially surrounded by a layer of brown, thick-walled cells which may serve as a barrier to its further nutrition. In such cases the results are not altogether unlike those obtained from a minute puncture or an insect sting.

5. If, however, the fungus attacks the host cells when they are nearly mature, it finds conditions more favorable for its development, because the cell sap furnishes more satisfactory food material and the cells are at the same time unable to respond to its presence. The result is a more vigorous development of the fungus and a rapid browning and drying of the host tissue.

6. The chlamydospores and sclerotial masses of the fungus are the probable agencies in carrying the disease through the winter.

7. Conidia have not been found on the host in the fall. They probably develop from sclerotia and pycnidia in the following

spring on apples that have lain on the ground over the winter, and thus become the agency in starting the disease the next season.

8. Spraying with Bordeaux is a preventive for the disease. Applications made late in June or early in July are as effective as those made earlier in the season.

9. By his references to the work reported from other stations the writer would not be understood to imply that the disease here under special consideration is *identical* with that described from any other section. However, he does not find anything in some of these reports to show that the Fruit Spot which is common in New Hampshire *may* not sometimes have been included along with the Fruit Pit in these descriptions. Further, this study leads the writer to the conclusion that because particular pathological conditions may originate without the presence of any foreign agency, this should not be taken as proof that very similar results may not be due to the presence of a parasitic fungus in the host tissue.

The writer wishes to acknowledge his indebtedness to Prof. H. H. Whetzel of Cornell University, Prof. W. J. Morse of the Agricultural Experiment Station of Maine, Dr. J. B. Dandeno of Michigan State Agricultural College, Prof. W. T. Macoun of the Central Experimental Farm, Ottawa, Canada, and Prof. C. P. Lounsbury of the Department of Agriculture, Cape Town, Africa, for specimens of spotted fruit.

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NEW HAMPSHIRE COLLEGE,
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Explanation of plates 29-35

PLATE 29

FIG. 1. Baldwin showing young stages of the Fruit Spot.

FIG. 2. Baldwin showing later stages of the Spot.

PLATE 30

FIGS. 1 and 2. Section through green spots of Baldwins showing a browning in the surface cells and pockets in the adjacent tissue. The fungus can be plainly seen in some of the largest pockets.

FIG. 3. Section through a green spot of a badly withered Baldwin, showing the thick-walled cells of the surface zone from which has later extended the large mass of shrunken brown cells beneath. The fungus can be seen in the pockets in this shrunken tissue.

FIG. 4. A cross-section of a thirty-four days old inoculation on a Baldwin. The mycelium may be seen in the needle path and also closely pressed against the walls of the withering cells.

PLATE 31

FIG. 1. A cross-section of an apple to show the location of the large bands of conducting tissue.

FIG. 2. Inoculations and punctures from a Baldwin after fifty days. The two at the right are inoculations.

FIG. 3. A section of an apple of which the cell tissue in the region of the vascular system is browned.

FIG. 4. A section of a typical colony from the walls of a ten days old culture of solution A. The lower side as shown in the plate was attached to the wall of the test tube.

PLATE 32

FIG. 1. A section through an early stage of a red spot. The opening through the epidermis has been enlarged by a breaking of the tissue in cutting. At the right the small regular cells of the lenticel are replaced by large thick-walled ones. In the pocket that extends into these from above may be seen the granular hyphae of the fungus.

FIG. 2. A late stage of the Fruit Spot, showing the stromatic layer of the fungus with a conical mass of immature sporophores above it.

FIG. 3. A later stage of the spot showing the stroma, the shrunken tissue beneath, and the sporophores pushing through the lenticel above.

FIG. 4. A lenticel of an apple with normal tissue beneath it.

FIG. 5. Fungus as grown in agar Petri cultures.

PLATE 33

FIG. 1. A section of one of the minute elevations from the stroma of a five months old flask culture. The pustule-like cavities are shown in the upper portion.

FIG. 2. A cross-section of a stroma from a liquid flask culture showing the layer of conidia and conidiophores.

PLATE 34

FIG. 1. Spores from cultures in solution A.

FIG. 2. The same spores after remaining twenty-one hours in Van Tieghem cells

FIG. 3. The same spores after forty-two hours.

FIG. 4. A drawing of a section through an early stage of a fruit spot.

PLATE 35

FIG. 1. Conidia from a pustule such as is shown in FIG. 3, PLATE 32.

FIG. 2. Spore production in solution A agar after four days.

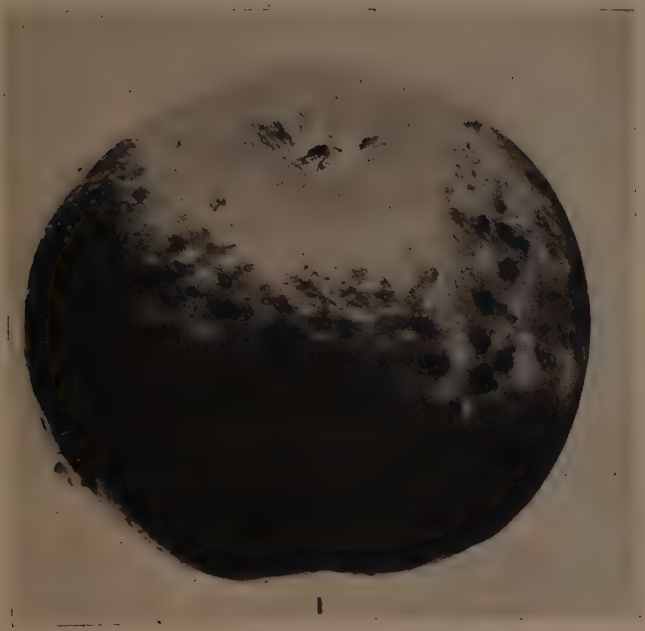
FIG. 3. Chlamydospores from an old culture.

FIG. 4. Germinating chlamydospores.

FIG. 5. Olive-brown hyphae from a six weeks old liquid culture after one day in a Van Tieghem cell.

FIG. 6. A similar hypha after two days in a Van Tieghem cell.

FIG. 7. A sketch of a portion of the vascular system of an apple.



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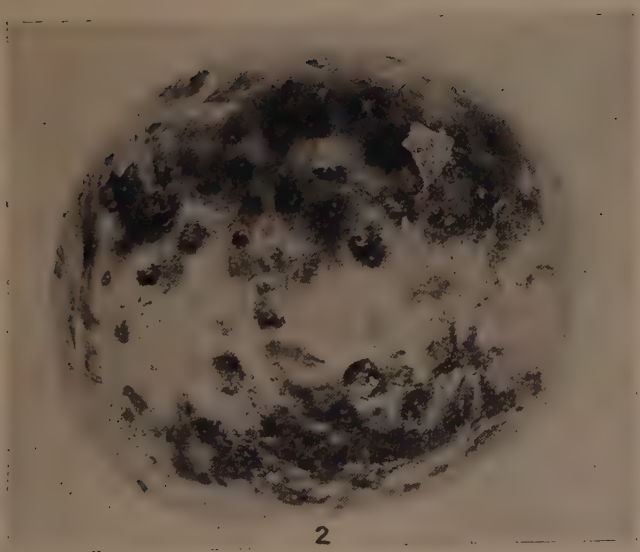

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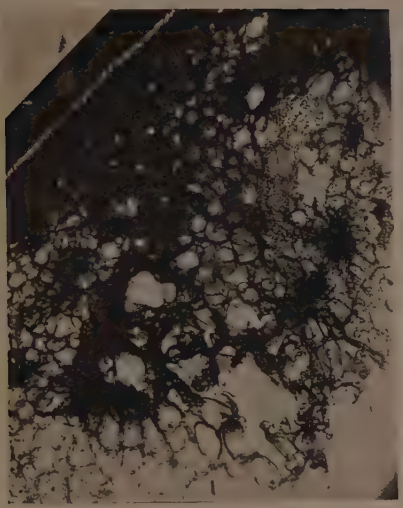
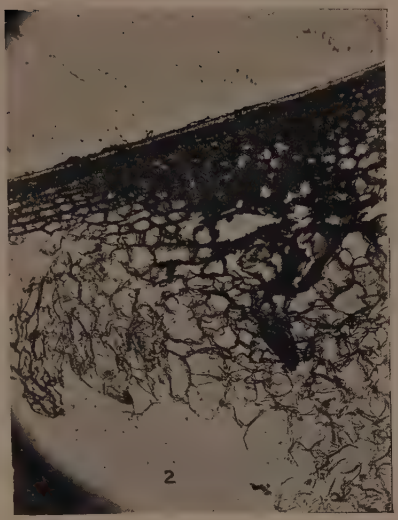
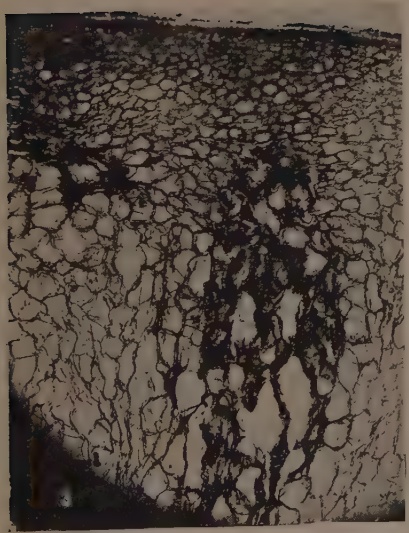
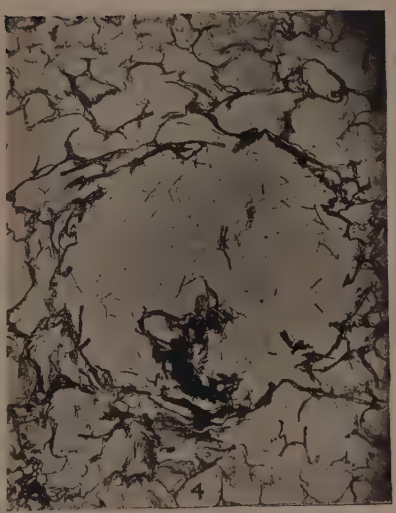
BROOKS, THE FRUIT SPOT OF APPLES









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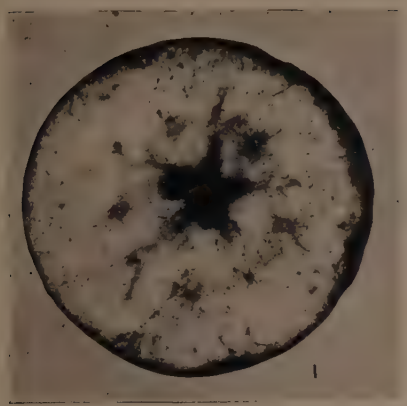

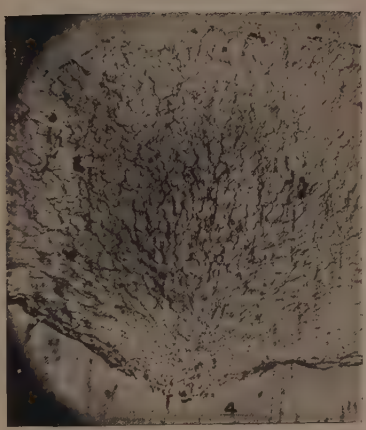

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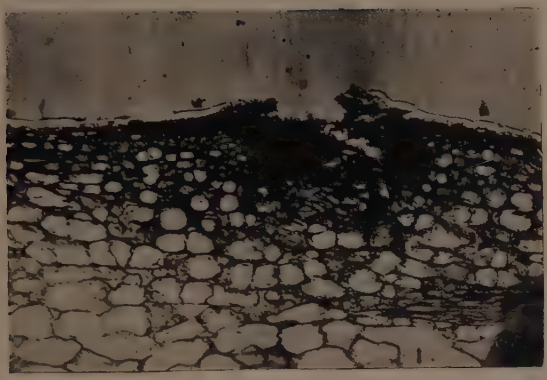

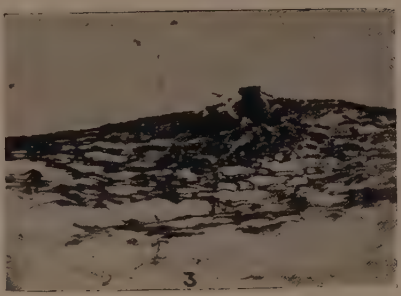

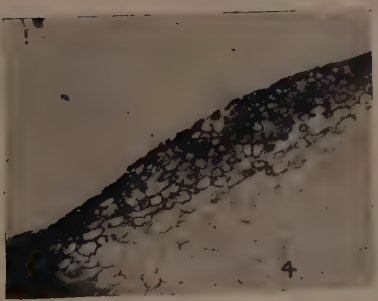

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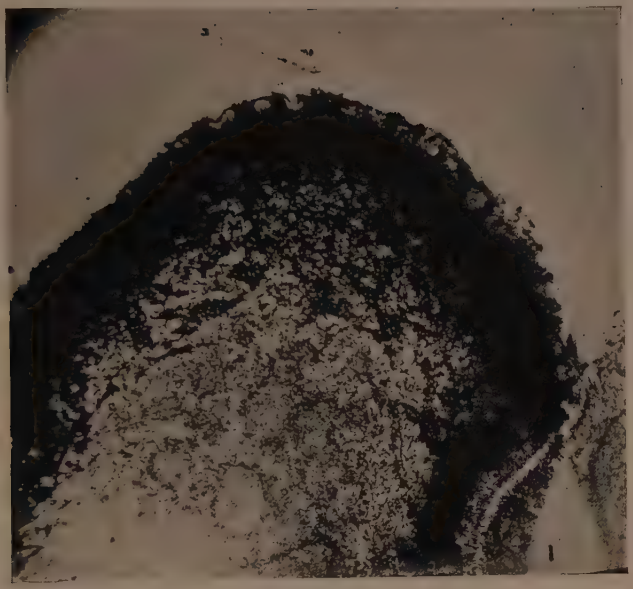

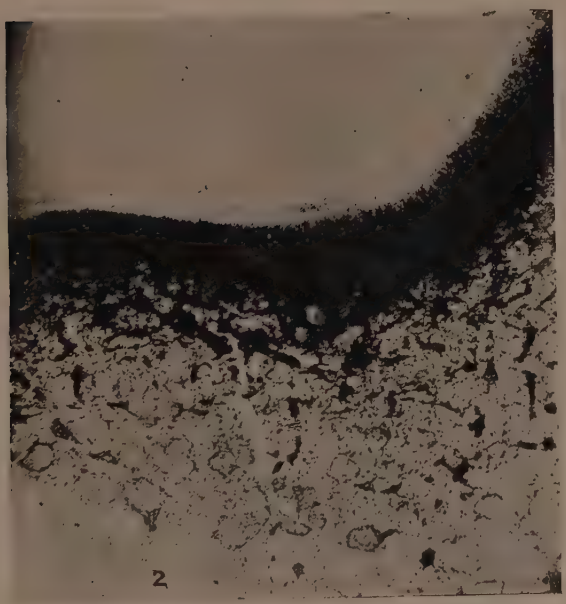

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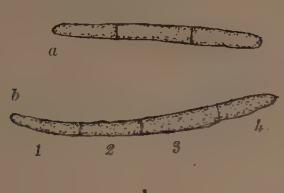

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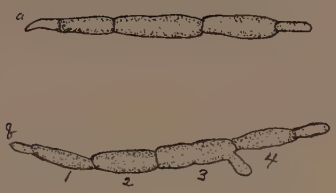

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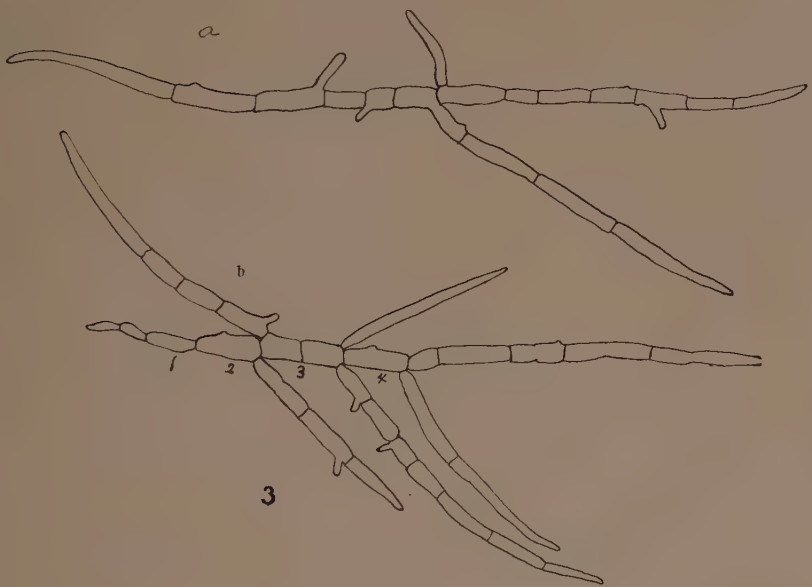

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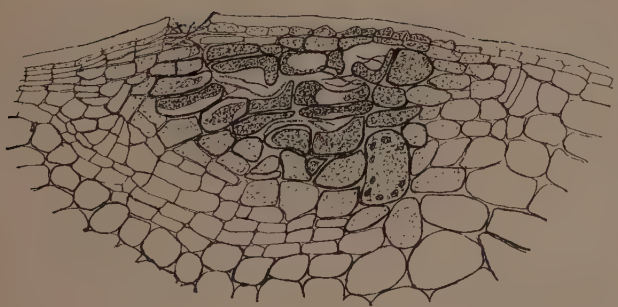

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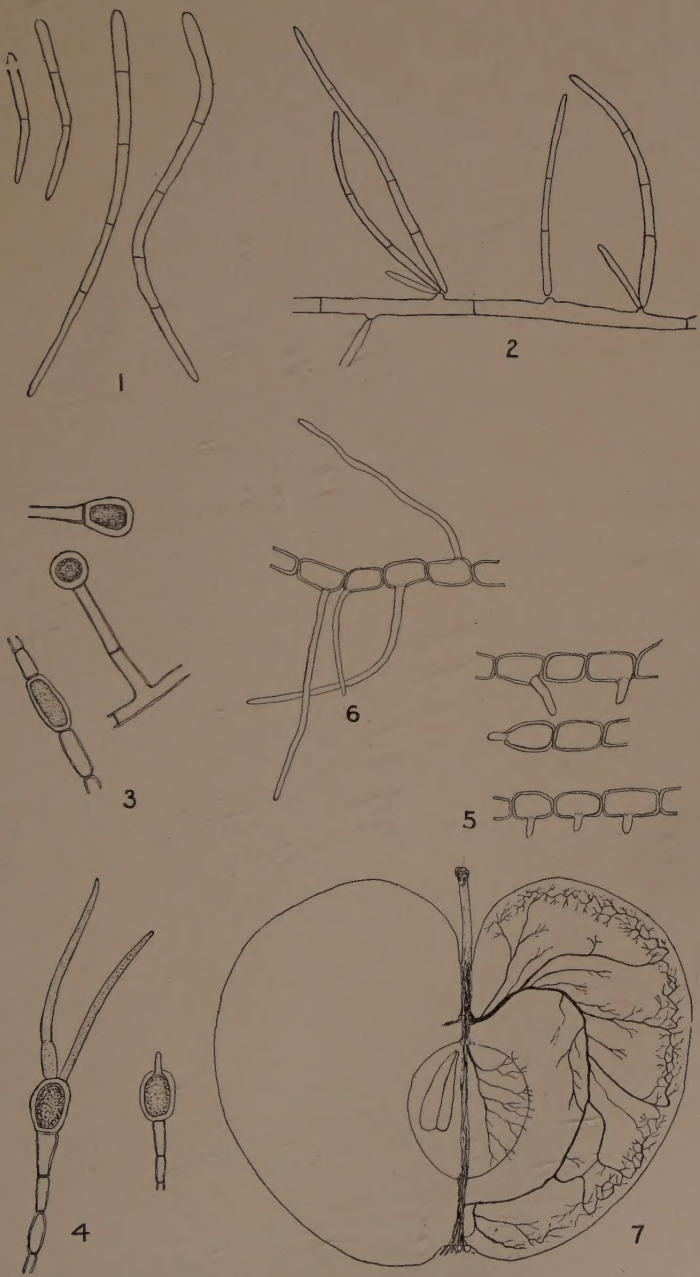

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